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An On-Chip Sensor for High-Throughput Profiling of Antimicrobial Resistance

Zhihao Liao
Iowa State University, zhihao@iastate.edu

An On-Chip Sensor for High-Throughput Profiling of Antimicrobial Resistance

by

Zhihao Liao

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The student author, whose presentation of the scholarship herein was approved by the program of study committee, is solely responsible for the content of this dissertation. The Graduate College will ensure this dissertation is globally accessible and will not permit alterations after a degree is conferred.

Iowa State University

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NOMENCLATURE

AMR	Antimicrobial Resistance
PCR	Polymerase Chain Reaction
PDMS	Polydimethylsiloxane
CDC	Centers for Disease Control
SOP	Standard Operating Procedure

ACKNOWLEDGMENTS

I would like to thank my major professor, Dr. Meng Lu, for his guidance and support throughout the course of this research.

In addition, I would also like to thank my friends, colleagues, the department faculty and staff for making my time at Iowa State University a wonderful experience. I want to also offer my appreciation to those who were willing to participate in my surveys and observations, without whom, this thesis would not have been possible.

Acknowledgments

ABSTRACT

Rapid and accurate determination of antimicrobial resistance (AMR) of pathogenic bacteria is a national priority owing to its vital importance to public health. We designed this project to develop an on-chip sensor system that is capable of characterizing bacterial drug susceptibility rapidly and inexpensively. First, in order to find an efficient polymerase chain reaction (PCR) method to detect the AMR genes, three different types on-chip microfluidic PCR systems were devised and fabricated by utilizing electric-heating, laser-heating based static chambers PCR system, and continuous flow PCR system. Second, we integrated DNA microarray and PCR on one chip to measure the expression level of a large number of AMR genes. The integration of PCR and DNA microarray on chip was fabricated by microfluidics technique. Electric-heating based static chambers PCR system consisted of a polydimethylsiloxane (PDMS) reaction chamber, a micro heater a cooler, a thermocouple, and a thermal controller. Electric-heating based continuous flow PCR system consisted of a thermocycler holder, three polyimide heaters, and 20 cycles Teflon tube. Laser-heating based static chambers PCR system consisted of a plasmonic absorber/thermocouple, a 3W laser, a solenoid shutter, a gasket chamber, and an Arduino controller. Each of PCR system was connected with microarray using microfluidic channels to form a whole integrated AMR sensor chip.

CHAPTER 1. INTRODUCTION

The antimicrobial resistance (AMR) of pathogenic bacteria is a global health threat[1]. The purpose of this project is to develop a AMR detection system that can be used for rapid characterization of bacterial drug susceptibility profile and thus reduce the excessive usage of antibiotics in healthcare. The Centers for Disease Control (CDC) estimates that antibiotic resistant bacteria cause at least two million illnesses and 23,000 deaths annually in the US[2, 3]. Due to the bacterial mobility in nature and their transformable cell membranes, it is very easy for one resistance gene to transfer from one bacterium to another and many bacteria thus acquire resistance toward multiple commonly used antibiotics[4-6]. Thus development of point-of-need diagnostics that rapidly distinguish between bacterial and viral infections and validation of diagnostics that rapidly determine the antibiotic-resistance profiles of bacteria of public health concern.

Currently, conventional culture-based methods require isolation of pathogens from clinical specimens, culture on media containing a range of concentrations of antibiotics, and determination of the minimum inhibitory concentration of each antibiotic. In most cases, a definitive result can be obtained more than 1-3 days when slow-growing pathogens need to be tested[7]. However, some infections are fatal if the treatment does not take place within 24 hours of the occurrence of the first symptoms[8, 9], forcing a physician to prescribe a broad-spectrum antibiotic. As a result, about 1/3 to 1/2 of all the antibiotics are unnecessary or incorrectly prescribed due to the slow turnover of the conventional culture-based AMR assay[2, 3]. To improve the conventional method, non-culture based methods have great potential to meet the requirements of rapid, accurate, high-throughput, and ease-of-use analysis. Thus, we designed

this method to rapidly and accurately determine of antimicrobial resistance (AMR) of pathogenic bacteria.

CHAPTER 2. THREE TYPES OF ON-CHIP MICROFLUDIC PCR SYSTEMS

In this two years, we devised and fabricated on-chip microfluidic PCR systems by utilizing three different methods: electric-heating, laser-heating based static chambers PCR system, and continuous flow PCR system[7, 10, 11].

2.1 Electric-heating Based Static Chambers PCR System

Design and the Fabrication Flow of the Proposed Sensor Chip

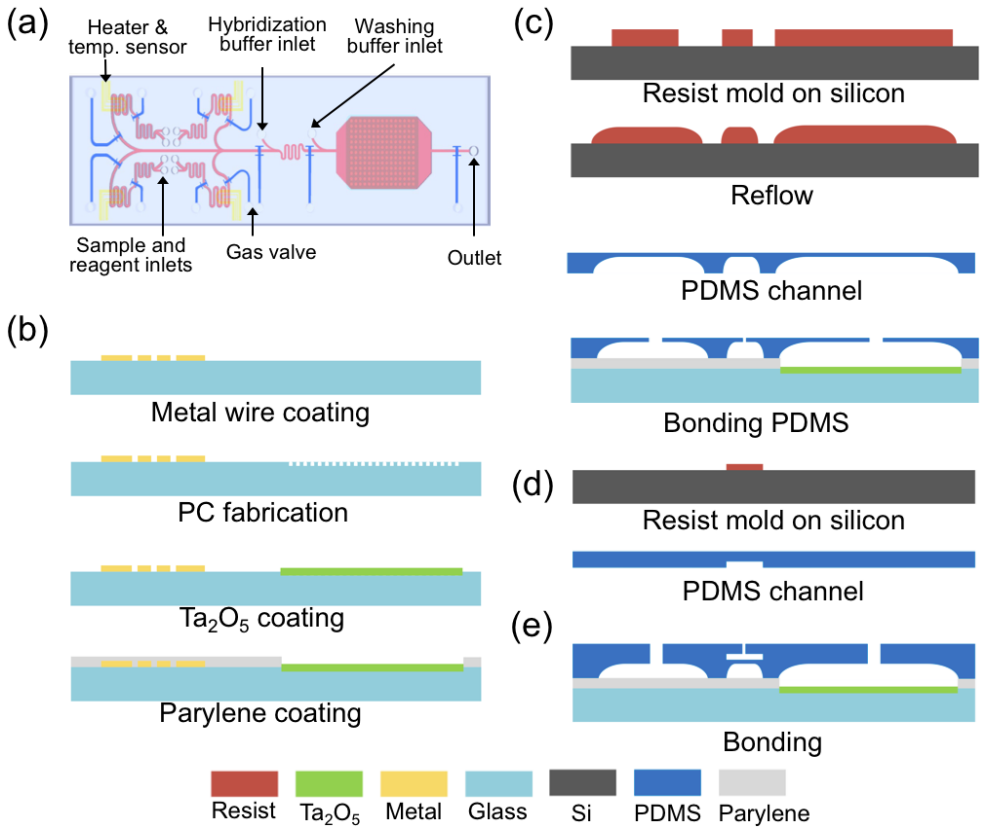

Figure 1. Schematic of the proposed lab-on-a-chip device built upon a microscope glass slide with four PCR reactors, a label-free DNA microarray, microfluidic channels, and microvalves.

Figure 1 (a). shows the top view of the chip with four sets of reactors. Each reactor will be used for multiplex PCR amplification.

Figure 1 (b). shows the fabrication of the metal wire heater/temperature sensor and the photonic crystal sensor.

Figure 1 (c). and Figure 1 (d). are molding PDMS for microfluidic and gas valve control layers.

Figure 1 (e). is the chamber and heater layer bonded by plasma cleaner.

Photomask Design

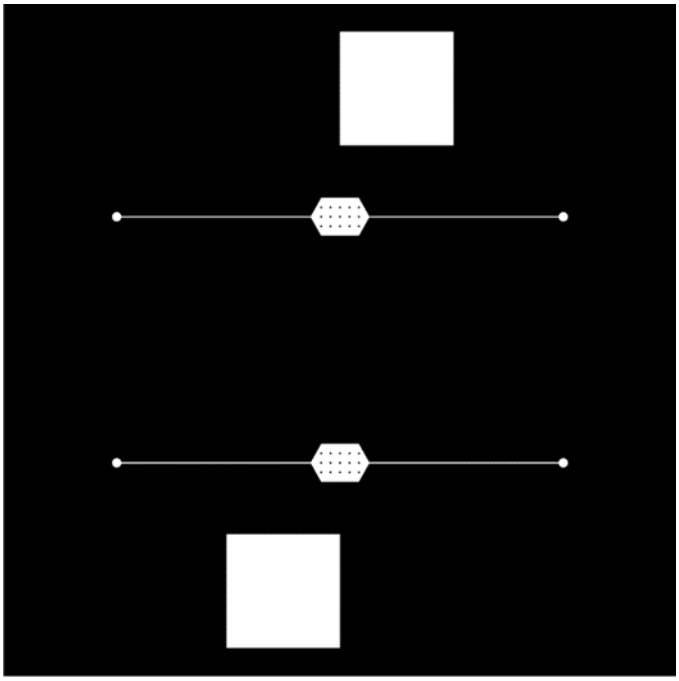

Figure 2 (a). Photomask for chamber

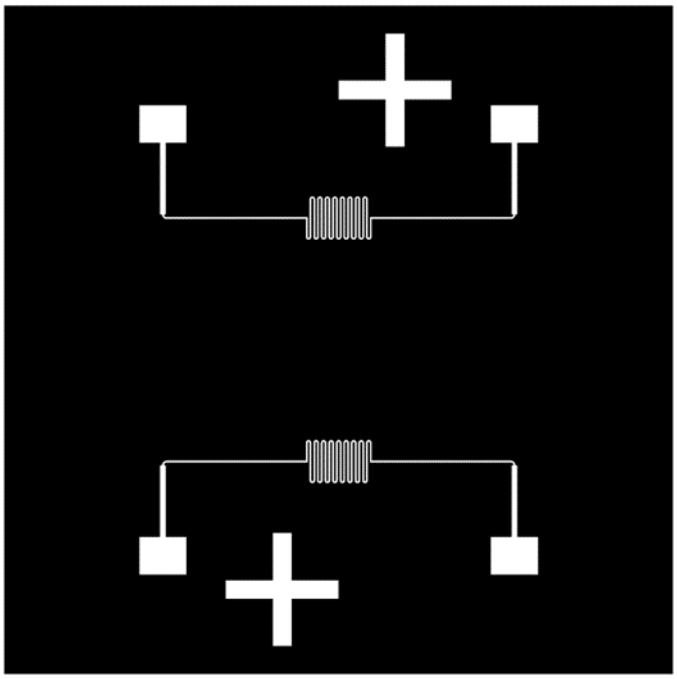

Figure 2 (b). Photomask for heater

Figure 2 (a). and Figure 2 (b). are our photomasks design.

The photomasks' size is: 20mm*20mm

Figure 2 (a). is the photomask for the chamber. Where:

Chamber: 4mm(L)*4mm(W)*60μm(Depth) =0.96 μL

Channel: 100μm(W) *60 μm(Depth)

Inlet & outlet: 1mm(Diameter)

Pillars in the chamber: 200μm(Diameter)

The pillars use for support the top of the chamber.

Setup

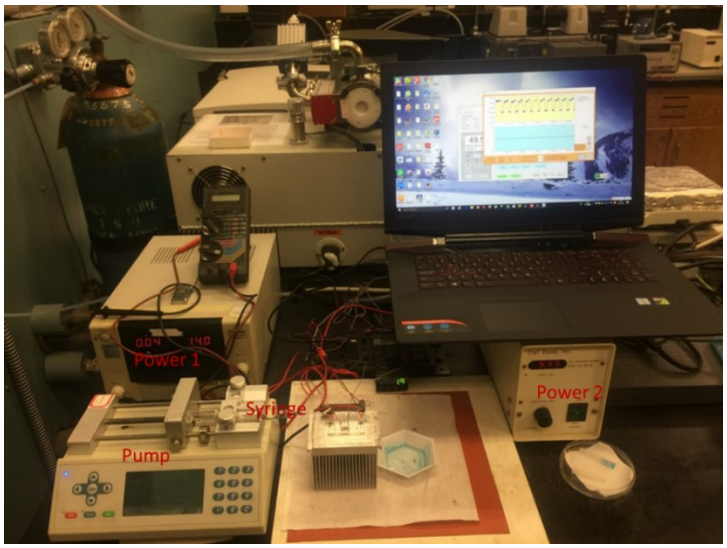

Figure 3. Whole system of static chambers PCR

Figure 3. shows the whole system. In this system, the thermocouple measured the temperature and gave the feedback to OMEGA™ controller at any time. When the temperature was lower than desired temperature, OMEGA™ controller turned the heater power on and turned the cooler power off. Similarly, when the temperature was higher than desired temperature, OMEGA™ controller turned the cooler power on and turned the heater power off.

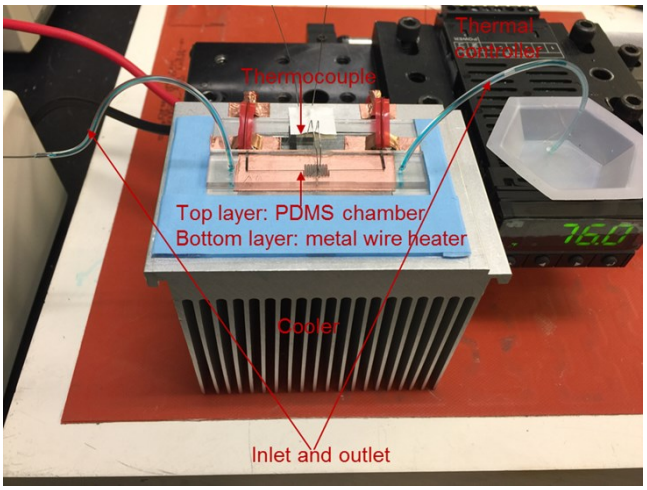

Figure 4. Microfluidic device of static chambers PCR

Figure 4. shows the microfluidic device. The PDMS chamber bonded with the heater by plasma cleaner. The electrodes connected with power, charged and heated the wire. The thermocouple inserted into the chamber, and connected with OMEGA™ controller. A cooler under the microfluidic device.

10 Cycles Test

Figure 5 (a). 10 cycles temperature rotation

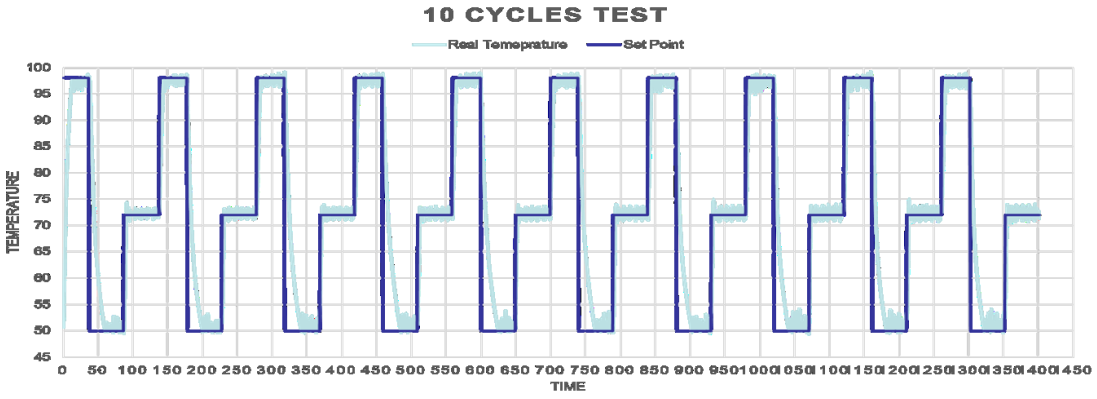

Figure 5 (b). 10 cycles temperature rotation

Figure 5(a). and Figure 5(b). show the 10 cycles temperature rotation. Each discrete cycle: heating to 98°C for 10 seconds, cooling to 50 °C for 20 seconds and heating to 72°C for 30 seconds.

In the figure, we can find out that temperature increases rapidly and achieves 98°C, but the temperature rate of decreasing is slower than the rate of increasing. Also after the temperature achieved our desired temperature, it was unstable.

2.2 Electric-heating Based Continuous Flow PCR System

Photomask Design

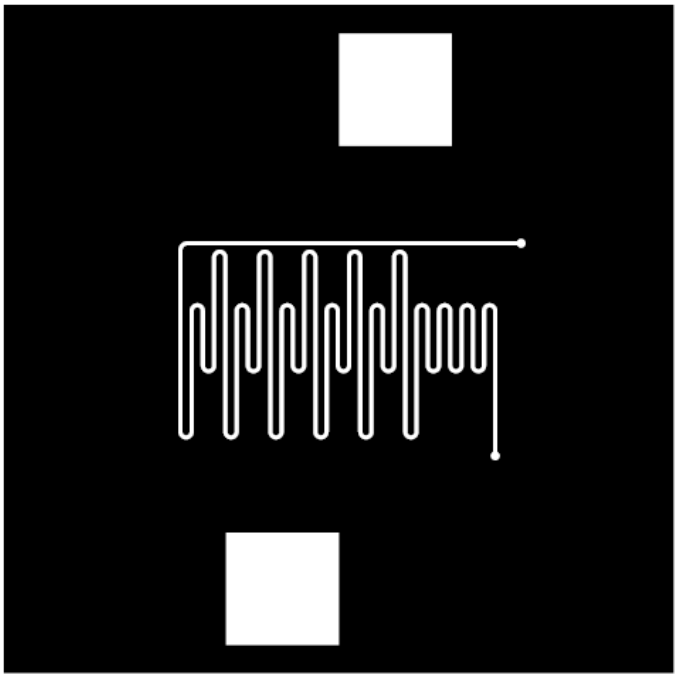

Figure 6 (a). Photomask for chamber

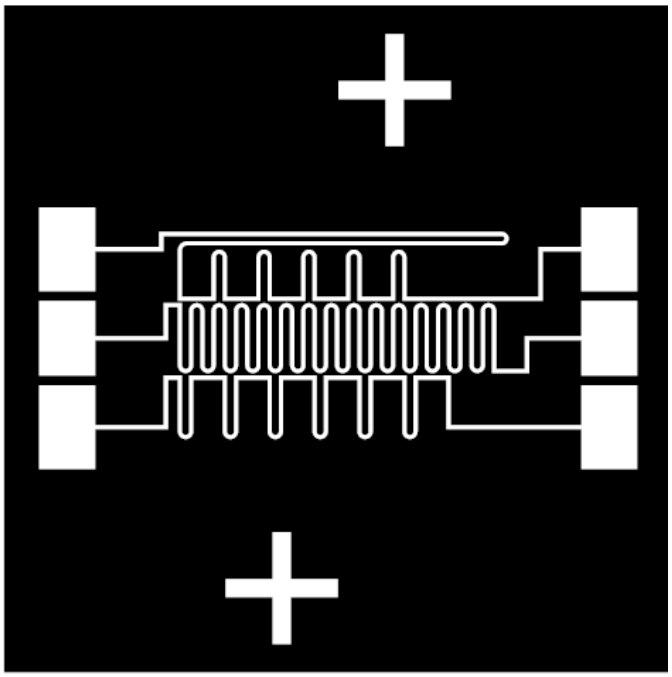

Figure 6 (b). Photomask for heater

Figure 6(a). and Figure 6(b). are our photomasks design.

The photomasks' size is: 20mm*20mm

Figure 6(a) is the photomask for the continue flow, including 6 Cycles. Where:

Channel: 100μm(W) *60 μm(Depth)

Inlet & outlet: 1mm(Diameter)

Figure 6(a). is the photomask for the heater, which has three different temperature zooms.

3D Continuous Flow PCR System

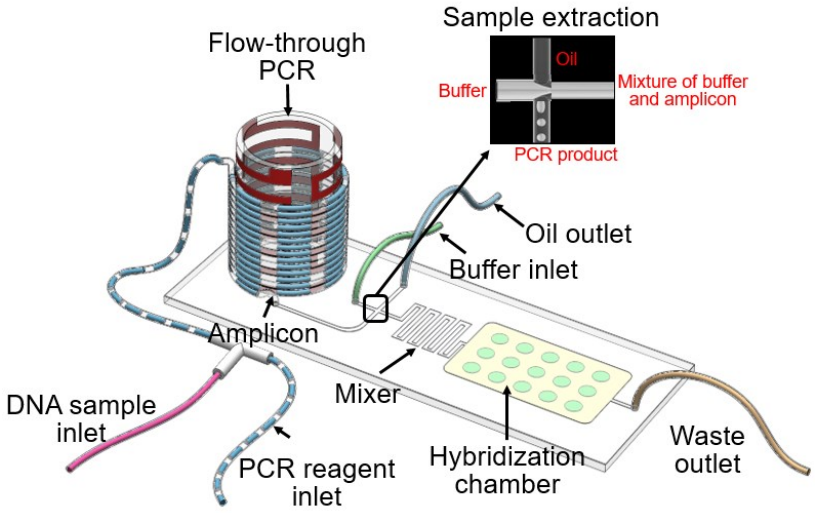

Figure 7. Schematic of 3D continue flow PCR systems

Figure 7. is the design of 3D continue flow PCR systems. This system includes: a flow-through thermocycler, 3 polyimide heaters, a hybridization chamber.

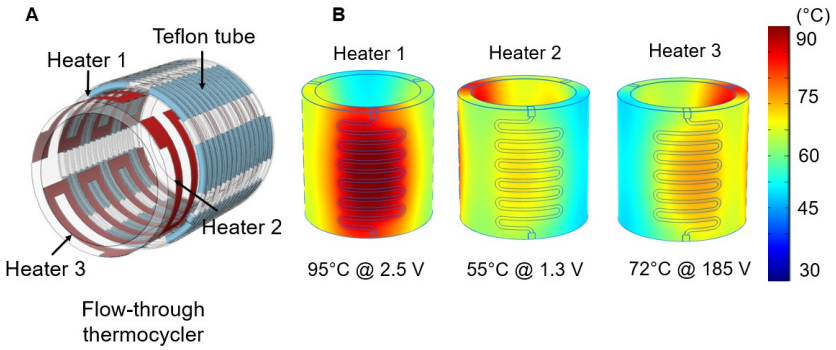

Figure 8. Temperature simulation for flow-through thermocycler

Figure 8. is the temperature simulation for flow-through thermocycler. The temperature interaction in adjacent temperature zones.

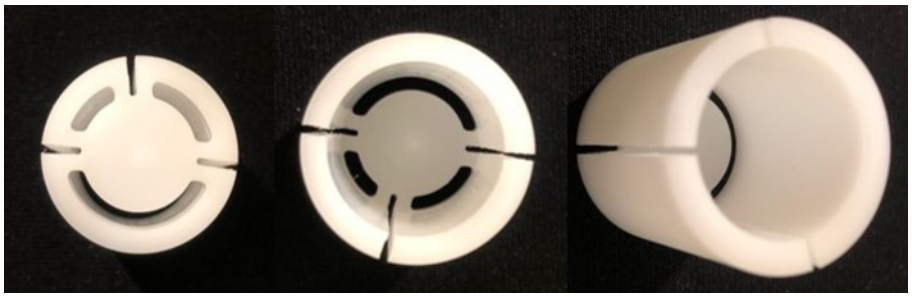

Figure 9. Flow-through thermocycler holder

Figure 9. is the different perspectives of the flow-through thermocycler holder.

In our design, the flow-through thermocycler holder made from DuPont™ Delrin® (Polyoxymethylene, POM).

We designed 3 slots for the holder to reduce the interaction between different temperature zones.

Each zone had different arc length. Since the sample in the tube had same flow rate, the different arc length determined the period in different temperature.

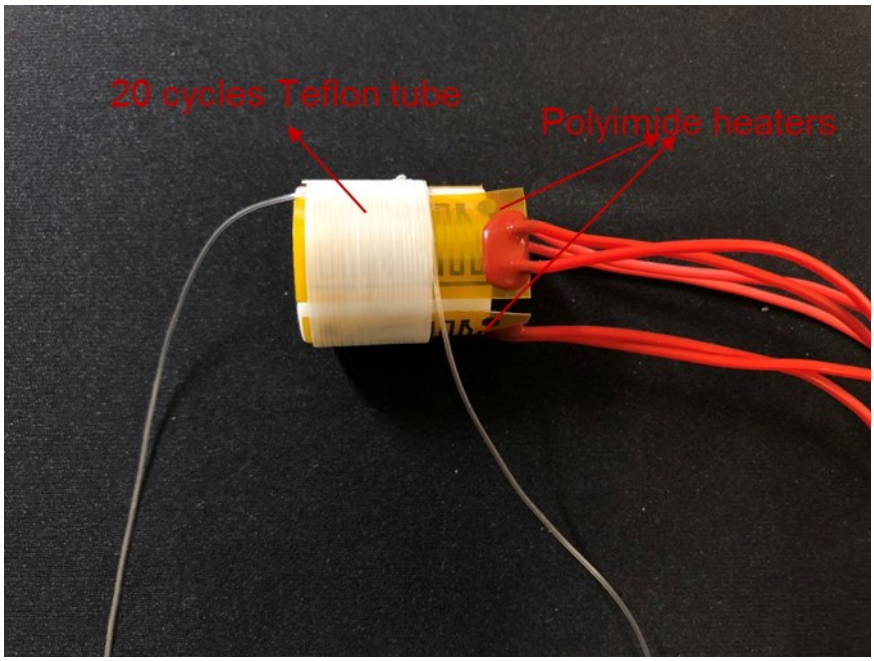

Figure 10. Flow-through thermocycler

We stuck the three polyimide heaters on three zones, wrapped with 20 cycles Teflon tube. Each heater set a constant temperature: 95°C, 50°C and 72°C. We controlled pump rate to get desired period of each part of PCR.

2.3 Laser-heating Based Static Chambers PCR System

Setup

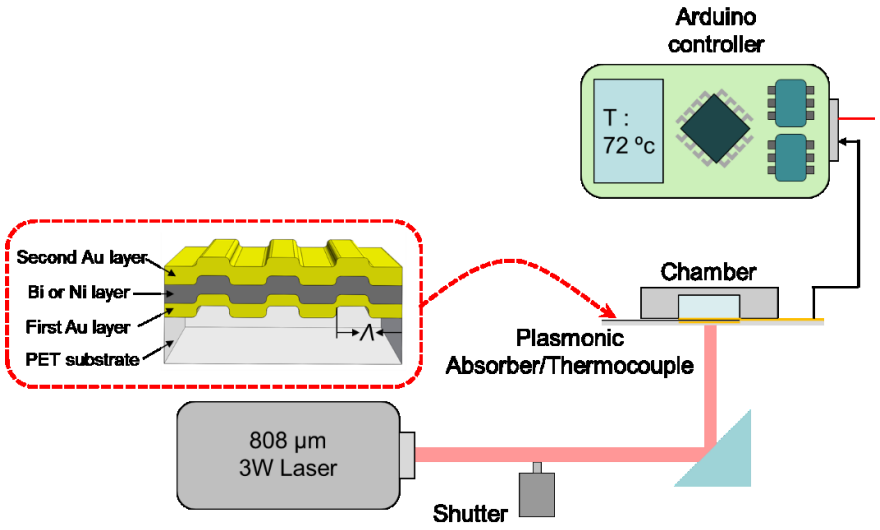

Figure 11. Schematic of laser-heating based static chambers PCR system

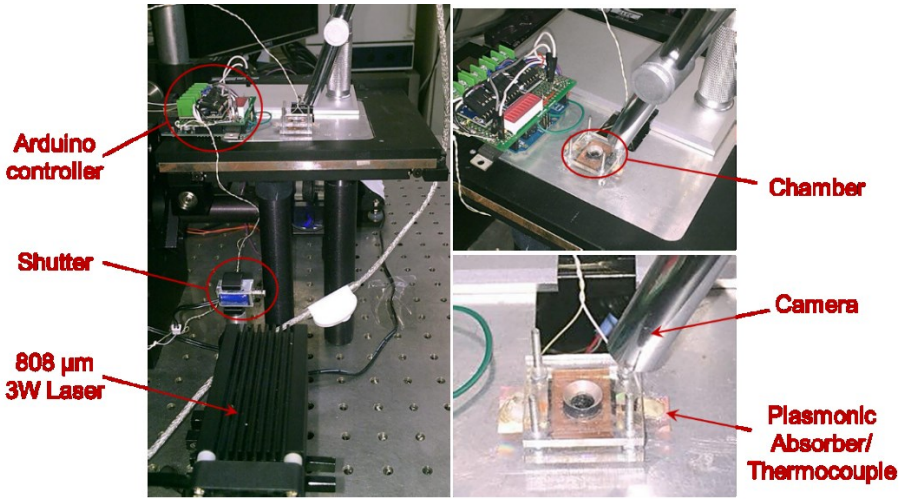

Figure 12. Whole system of laser-heating based static chambers PCR

The Figure 11. and Figure 12. show the whole system of laser-heating based static chambers PCR.

We designed plasmonic absorber/thermocouple, which had three metal layers on PET substrate. The first Au layer and Bi or Ni layer worked as a thermocouple, which converted heat to current flow by Seebeck coefficient contrast. The second Au layer on the top with plasmonic grating, which absorbed incident light and converted incident light to heat.

In this system, the thermocouple measured the temperature and gave the feedback to Arduino controller at any time. When the temperature was higher than desired temperature, Arduino controller sent a signal to solenoid shutter to block the laser source. Similarly, when the temperature was lower than desired temperature, the Arduino controller sent a signal to solenoid shutter to open the laser source.

Plasmonic Absorber/Thermocouple Calibration

Table 1

Temperature	Arduino Output
41	178
53.1	199
61.5	214
64.2	218
69.9	222
76.8	240
87.5	260
95.3	276
99.9	280

Table 1 records the temperature vs. Arduino output when we did the calibration.

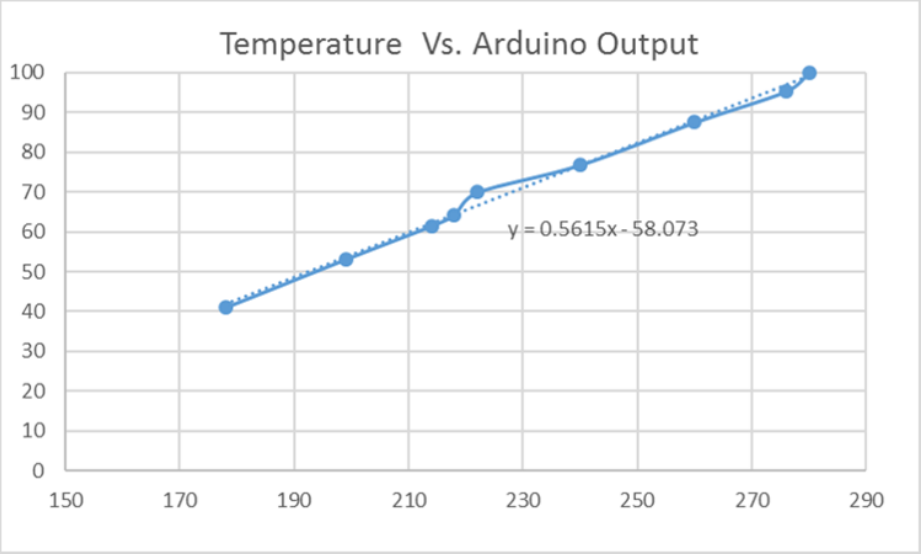

Figure 13. Temperature Vs. Arduino Output

Figure 13. shows the calibration of plasmonic absorber/thermocouple, temperature vs. Arduino output was almost linear:

$$Temperature = 0.5615x - 58.073,\ where\ x\ is\ Arduino\ output$$

CHAPTER 3. DNA MICROARRY ANALYSIS

To do feasibility study of the antimicrobial resistance of pathogenic bacteria, we bought Agilent G2534A Microarray Hybridization Chamber to detect the common resistance genes.

Determination of Antimicrobial Resistance of Genes by DNA Microarray

DNA Preparation

Extracted 12 genes from E.coli genome, amplified the DNA fragments covering the Hybridization probes by the multiplex-PCR conditions. Purified the PCR products by Zymo-DNA Clean & Concentrator™ Kit, and label the DNA by the Label IT Cy5 Nucleic Acid Labeling Kits.

DNA Hybridization

Added 0.1 volume of Denaturation Reagent D1 to the labeled sample. Mix well and incubate for 5 minutes at room temperature. Added 0.1 volume of Neutralization Buffer N1. Mix well and incubate on ice for 5 minutes. Concentrated the Cy3-labeled samples.

Pre-hybridized the required microarray slides for 45 minutes at the hybridization temperature by a pre-hybridization buffer containing 5X SSC, 0.1% SDS, and 1% BSA.

Dipped slides in dH_2O and quickly dried by compressed air.

Diluted the Cy3-labeled samples in the hybridization buffer: 30% formamide, 0.5% SDS, 6xSSC, 5xDenhardt's solution.

Applied the mixture to a hybridization chamber and incubated at 43°C for 16 h in an oven.

Washed the slides sequentially in Solution A (1x SSC, 0.2%SDS) for 3 min, Solution B (0.2x SSC) for 3 min, and solution C (95% alcohol) for 1.5 min. Dried the slides under a gentle airstream before they were scanned.

CHAPTER 4. RELATED WORK

In addition to the above work, I also did the following related research to help my future work for colleagues.

4.1 Evaporation in Gasket Chambers

Setup

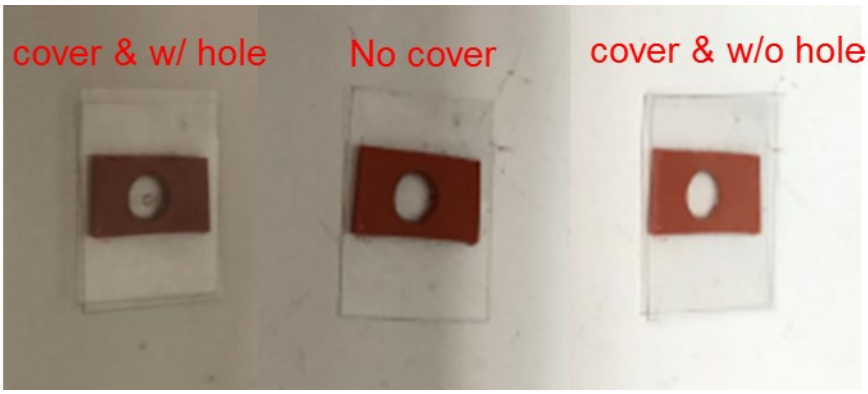

Figure 14. Gasket chamber on PET substrate, w/wo PET cover

I fabricated three devices:

1. Gasket chamber on PET substrate (Figure 14. Middle).
2. Gasket chamber on PET substrate, covered by other PET plate (Figure 14. Right).
3. Gasket chamber on PET substrate, covered by other PET plate with hole (Figure 14. Left).

Experiment

Stuck thermocouple under the PET substrate, put the device on hotplate. Adjusted the hotplate temperature to 100°C.

Dropped 1 mL water in Gasket chamber to each device by pipette.

Recorded evaporation time and observed the bubble in the chamber.

Similarly, I prepared 20% glycerol.

Dropped 1 mL 20% glycerol in Gasket chamber to each device by pipette.

Recorded evaporation time and observed the bubble in the chamber.

Table 2

Cover	Hole	Glycerol	t	Bubble
×	×	×	5:30	×
√	×	×	7:30	√
√	√	×	8:00	√
×	×	√	> 8:30	×
√	×	√	> 8:30	×
√	√	√	> 8:30	×

In Figure Table 2, we concluded that covered with PET plate and glycerol adding had the function of reducing evaporation and bubble.

4.2 Heating Characterization for Whatman™ Paper

Whatman™ Paper Painted by Graphene or Graphite (Pencil)

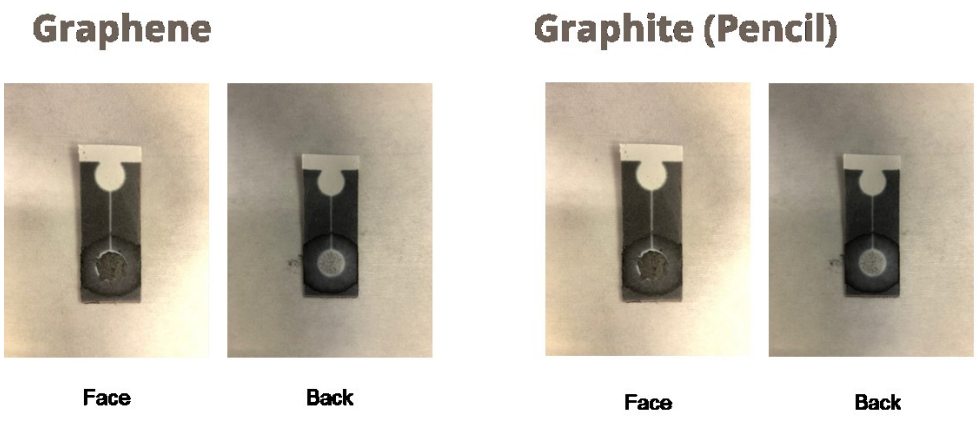

Figure 15. Whatman™ Paper Painted by Graphene or Graphite (Pencil)

The Whatman™ Paper printed by wax.

Stirred graphene and dropped on the chamber of Whatman™ Paper. Also, painted graphite on Whatman™ Paper by pencil.

Figure 15. shows the Whatman™ Paper face and back after painting graphene and graphite.

Heating and Evaporation

Heated the chamber after painting graphite and graphene by laser. Recorded the time of the temperature to 100°C. Also tested the time for completing evaporation, whose chambers were covered by PET and tape.

Table 3

Material & Method	Time
Graphite	14s
Graphite (With Water)	54s
Graphene	13s
Graphene (With Water)	56s
Graphite (Water covered by PET and tape)	49s
Graphene (Water covered by PET and tape)	50s

Table 3 shows the time comparison with different materials (graphite and graphene) and different methods.

4.3 SOP for Spin Coater

Standard Operating Procedure for Spin Coater

I summarized and formulated the Standard Operating Procedure(SOP) for spin coater in our lab.

SOP for Spin coater

Warming:
Because of water and chemicals can get pulled into the vacuum line.
Wafers should **NOT** be wet or have sticky chemicals of any kind on the back side.
Must **NEVER** spray solvents onto the chuck while it is on the tool.
When cleaning the bowl, a dummy wafer or glass should **ALWAYS** be clamped onto the chuck.

0. For easy cleaning after coating, covered all chamber and lid by foil paper.
1. Plug in pump power (right side of the hood).
2. Turn on the compress air switch (orange, right side of the hood).
3. Open the lid and place your substrate on the center of vacuum chuck.
   Your substrate **MUST COMPLETELY** cover the "O-ring" (a black ring on the chuck).
   If your sample smaller than samples 2", use adapter chuck (around the spinner in a plastic bag) for small samples.
4. Set the required program.
   Before editing a new program, please check "Commonly Used Program List". You can pick up an existing program which same as your desired, or select an unassigned program to edit. Also, if you will often use a program, you can assign the program, and record the program's name and data on "Commonly Used Program List". After you don't often use the program, please remove it from the list.
   - If editing an existing program, press **"PROGRAM SELECT"** key to select the desired program.
   - If creating a new program, press **"PROGRAM SELECT"** key to select an unassigned program.
     1) Press the **"F1"** key. The "PGM" message will appear in the mode area of the display to indicate that the unit is in "programming" mode.
     2) Use **"ADD STEP"** & **"DEL STEP"** keys to select or remove the number of steps necessary in the program. Up to 51 steps can be programmed.
     3) Use "**CURSOR ←(L) & →(R)"** keys to position the cursor over the value to be changed.
     4) Use **"VALUE ↑(UP) & ↓(DOWN)"** keys to change set point values.
     5) Use **"STEP"** key to advance to the next step.
     6) Press **"F1"** key to return to "run" mode.
5. Turn the vacuum on by pressing **"VACUUM"** button on panel.
6. Dispense the solution on the substrate.
7. Close the lid and press **"RUN/STOP"** key.
8. Remove your sample and put dummy wafer or glass clamped onto the chuck.
9. Remove foil paper and wet a wipe with acetone clean all the chamber and lid.
10. Plug off pump power and turn off the compress air switch.

CHAPTER 5. SUMMARY

In this project, we devised and fabricated on-chip microfluidic PCR systems by utilizing three different methods: electric-heating, laser-heating based static chambers PCR system, and continuous flow PCR system; Developed and assembled temperature measurement and control systems for different methods of PCR systems by utilizing thermocouple, thermo-controller, OMEGA™ controller and Arduino microcontroller; Developed plasmonic heating and thermocouple for laser PCR systems; Detected antimicrobial resistance genes using a label-free DNA microarray.

We encountered some issues in our previous research. The first problem is how to reduce evaporation and bubbles in the samples. Also, how to keep the temperature constant in temperature controlling phase is another problem.

Some related work is included: Evaporation in gasket chambers; heating characterization of Whatman™ paper; formulating the standard operation procedure (SOP) for spin coater in our lab. This related work will be used as a solid foundation for the future research, such as paper-based point-of-care testing in disease diagnostics.

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